

FCS10- Procedure for Chemical Spot Tests

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1. Scope

- 1.1. To describe the chemical screening procedures, commonly referred to as chemical spot tests, for preliminary or screening tests of controlled and non-controlled substances.

2. Background

- 2.1. To establish the practices for documenting the examination of evidence to conform to the requirements of the Department of Forensic Sciences (DFS) Forensic Chemistry Unit (FCU) *Quality Assurance Manual*, the accreditation standards under ISO/IEC 17025:2005, and any supplemental standards.

3. Safety

- 3.1. Read Material Safety Data Sheets to determine the safety hazards for chemicals and reagents used in the standard operating procedures.
- 3.2. Wear personal protective equipment (e.g., lab coat, gloves, mask, eye protection), when carrying out standard operating procedures.
- 3.3. Note, when handling items that are suspect to contain fentanyl, or fentanyl analog, perform accessioning in fume hood is advised when possible. If the substance is powder or has the potential for aerosolization, then the item shall be accessioned and sampled in the fume hood.

4. Materials Required

- 4.1. Spot plates, pipettes, or other appropriate containers/items
- 4.2. Reagents appropriate to the specific chemical spot tests.

5. Standards and Controls

- 5.1. Each reagent must be labeled with the name of the solution or reagent. The analyst's initials and the date prepared must be recorded on the label or in an appropriate logbook.
- 5.2. Freshly prepared reagents will be quality tested with known reference standards and the results recorded in a retrievable logbook.
- 5.3. Unless otherwise specified, performance of reagents will be verified monthly and the results of the checks placed in a logbook. If the reagent has not been used for a month or more, it must be checked using a standard (and the results of the check logged) before its use with case samples.
- 5.4. It is the responsibility of the analyst to determine if reagents are working properly, and to periodically quality-test them and document the results. Reagents which do not respond appropriately to quality testing will be discarded.

6. Calibration

- 6.1. Not applicable

7. Procedures

7.1. MARQUIS TEST

7.1.1. Reagents/Chemicals

- Conc. Sulfuric acid
- Formaldehyde Solution (approx. 37% Formaldehyde)

7.1.2. Marquis Reagent

- Marquis Reagent: Add 5 to 10mL of approx. 40% formaldehyde solution to 100mL of concentrated sulfuric acid
- Store in a cool, dry place
- Quality-test reagent with heroin or acetylsalicylic acid.

7.1.3. Procedure

- Add a couple drops to a test container
- Add a small amount of sample to test
- Observe color change (or changes)

Note: If during the period of observation multiple color transitions are observed, the Analyst shall record all relevant colors observed, as determined by the Analyst.

7.1.4. Interpretation (color test acceptance criteria)

- **Purple** may indicate presence of Heroin
- **Orange - Brown** may indicate presence of Methamphetamine
- **Yellow to Brown to Violet** may indicate presence of Oxycodone
- **Orange - Brown** may indicate presence of Amphetamine
- **Pink – Red** may indicate presence of Acetylsalicylic Acid
- **Salmon** may indicate presence of Cocaine

The color which appears must be documented on the examination worksheet.

7.2. COBALT THIOCYANATE

7.2.1. Reagents/Chemicals

- Cobalt thiocyanate
- Deionized or better water (H₂O)
- Dilute HCl, acetic acid, or other acid

7.2.2. Preparation:

- 2% Cobalt Thiocyanate Reagent: Dissolve 2 g cobalt thiocyanate in 100 mL water (deionized grade or better).
- Store in a cool, dry place
- Quality-test reagent with procaine hydrochloride or cocaine standard.

7.2.3. Procedure:

- Combine a small amount of sample with the reagent (pink color). If a positive result is obtained (pink turns to blue color), the analyst may stop and record any observations.
- If the color remains pink, add a few drops of acetic acid to the sample and record any observations.

7.2.4. Interpretation (color test acceptance criteria)

- Upon mixing sample with reagent a **blue color results** (positive, +, result), indicating cocaine or a similar substance may be present.
- Note – if no color change is observed upon mixing with cobalt thiocyanate, but with addition of acetic acid the **pink color turns blue, this indicates cocaine base may be present.**

7.3. SODIUM NITROPRUSSIDE TEST

7.3.1. Reagents/Chemicals

- Sodium Nitroprusside
- Purified H₂O
- Acetaldehyde
- 1M Sodium Hydroxide (NaOH)
- 1M Sodium Carbonate (Na₂CO₃)

7.3.2. Preparation:

- Dissolve 0.09 g sodium nitroprusside in a mixture of 1 mL acetaldehyde and 9 mL water.
- Quality-test reagent with a methamphetamine standard.

7.3.3. Procedure:

- Combine a small amount of sample with a few drops of SNP Reagent.
- Add a few drops of 1M Na₂CO₃ (or NaOH) to the sample.
- Record any observations.

7.3.4. Interpretation (color test acceptance criteria)

- A positive indication on the worksheet means a reaction that forms a **blue color (+)** which indicates the possible presence of secondary amines, such as methamphetamine.

7.4. FERRIC CHLORIDE TEST

7.4.1. Reagents/Chemicals

- Ferric Chloride, $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$
- Deionized water or better
- 5% Ferric Chloride Reagent: Dissolve 0.83 g $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ in 10 mL water.
- Quality-test reagent with GHB or aspirin.

7.4.2. Procedure:

- Combine a small amount of sample and a few drops of 5% Ferric Chloride reagent.
- Record any observations.

7.4.3. Interpretation (color test acceptance criteria)

- A reaction that forms an orange-brown color indicates the possible presence of GHB.
- A reaction that forms a dark purple color indicates the possible presence of salicylates.
- A reaction that forms a bluish-gray color indicates the possible presence of acetaminophen.
- The resulting color must be indicated on the examination worksheet.

7.5. COBALT NITRATE TEST

7.5.1. Reagents/Chemicals

- Cobalt nitrate
- Isopropylamine
- 95% ethanol

7.5.2. Procedure:

- 1% Cobalt nitrate in ethanol reagent: Add 1 g cobalt nitrate to 100 mL ethanol.
- 5% Isopropylamine in ethanol reagent: Add 5 g isopropylamine to 100 mL ethanol.
- Quality-test reagent with a gamma-hydroxybutyrate (GHB) or barbiturate standard.

7.5.3. Procedure:

- Combine a small amount of sample and a few drops of 1% cobalt nitrate in ethanol reagent.
- Record any observations.
- Add a few drops 5% isopropylamine to sample.
- Record any observations.

7.5.4. Interpretation (color test acceptance criteria)

- A **purple color** upon addition of 1% cobalt nitrate in ethanol indicates the possible presence of gamma-hydroxybutyrate (GHB+).
- A **purple color** which only forms after also adding 5% isopropylamine in ethanol indicates the possible presence of barbiturates (Barb+).

NOTE: Known False Positives – Acetonitrile gives a positive (blue) result upon addition of cobalt thiocyanate. Care should be taken to ensure that neat samples are tested (or samples extracted in other solvents) and not acetonitrile extracts to avoid false positive results.

7.6. p-DMABA TEST

7.6.1. Reagents/Chemicals

- 95% Ethanol
- p-Dimethylaminobenzaldehyde
- Conc. HCl

7.6.2. Procedure:

- p-DMABA reagent: Dissolve 0.1 g p-dimethylaminobenzaldehyde in 9.5 ml ethanol. Add 0.5 ml conc. HCl.
- Quality-test reagent with benzocaine, procaine, or LSD.

7.6.3. Procedure:

- Combine a small amount of sample and a few drops of p-DMABA reagent.
- Record any observations.

7.6.4. Interpretation (color test acceptance criteria)

- A reaction which forms a **bright yellow color** indicates the possible presence of procaine or benzocaine.
- A reaction which forms a **purple color** indicates the possible presence of LSD.
- The resulting color must be indicated on the examination worksheet.

7.7. WEBER TEST

7.7.1. Reagents/Chemicals

- Fast Blue B (o-Dianisidine bis(diazotized) zinc double salt)
- Conc. HCl
- Deionized water, or better

7.7.2. Procedure:

- 0.1% Fast Blue B: Dissolve 0.1 g Fast Blue B in 100 mL H₂O.

Note - Prepare this reagent fresh and quality-test with psilocin or a sample of mushroom shown to contain psilocin before use.

7.7.3. Procedure:

- Combine a small amount of sample or methanol extract of the sample and a few drops of 0.1% Fast Blue B wait approximately one minute.
- Add one volume of conc. HCl.
- Record any observations.

7.7.4. Interpretation (color test acceptance criteria)

- A positive reaction for psilocin is indicated if a red color forms after adding the Fast Blue B reagent, and if after adding HCl the color changes to blue (appropriate color sequence = Weber+).

7.8. DUQUENOIS-LEVINE TEST

7.8.1. Reagents/Chemicals

- Vanillin
- 95% Ethanol
- Acetaldehyde
- Conc. HCl
- Chloroform
- Petroleum ether

7.8.2. Preparation of the Duquenois Reagent:

- Add 2 g vanillin to bottle (>100mL size)
- Add 2 mL acetaldehyde
- Add 100 mL 95% ethanol
- Store in a cool, dry place
- Quality-test reagent with marijuana.

7.8.3. Procedure:

- Place small amount of plant material in testing container. Either proceed directly to next step or extract plant material with petroleum ether. Discard plant material and evaporate to dryness. This step is optional and may be performed on difficult samples or at the discretion of the analyst.
- Add approximately one milliliter of the Duquenois reagent.
- Add approximately one milliliter of concentrated Hydrochloric Acid and wait up to a minute for color to appear. (It is not necessary to wait as long with the extract.)
- Observe color.
- Add approximately two milliliters of chloroform and wait for layers to form.
- Record any observations (color changes and layers).

7.8.4. Interpretation: (color test acceptance criteria)

- A **blue to violet color** after the addition of HCl to the mixture of Duquenois reagent and plant material or extract is a positive reaction and indicates the possible presence of cannabinoids.
- After adding chloroform and mixing, a **purple color in organic (lower) layer** is a positive reaction for the possible presence of cannabinoids.
- A positive result indicates that components (cannabinoids, including THC) unique to marijuana, marijuana residue, or hashish are present.
- A positive (or +) indication on the worksheet means test resulted in a purple/violet color after addition of hydrochloric acid and Duquenois reagent, and that the chloroform layer also yields purple color.

NOTE: Known False Positives: Plants patchouli, cypress, and eucalyptus.

7.9. FORMALDEHYDE-SULFURIC ACID TEST

7.9.1. Reagents/Chemicals

- Conc. Sulfuric Acid
- Formaldehyde Solution (*i.e.*, 37% Formaldehyde)

7.9.2. Preparation:

- Formaldehyde-Sulfuric Reagent: Add 6 volumes of formaldehyde solution to 4 volumes conc. Sulfuric acid. Keep the pipette tip just below the surface during the addition; stir and (if necessary) cool the mixture.
- Quality-test reagent with a known sample of benzodiazepines.

7.9.3. Procedure:

- Combine a small amount of sample and a few drops of formaldehyde-sulfuric test reagent in a test tube.
- Heat to approximately 100deg C for approximately a minute.
- Record any observations.

7.9.4. Interpretation (color test acceptance criteria)

- Benzodiazepines generally give an orange color.
- The resulting color must be indicated on the examination worksheet.

7.10. JANOVSKY TEST

7.10.1. Reagents/Chemicals

- m-dinitrobenzene
- Potassium hydroxide
- Absolute ethanol

7.10.2. Preparation:

- Janovsky Solution A: 2% m-dinitrobenzene: Add 2 g m-dinitrobenzene to 100 mL absolute ethanol.
- Janovsky Solution B: 5N potassium hydroxide: Add 28.05 g potassium hydroxide to 100 mL purified
- Quality-test reagents with Ketamine and/or Flunitrazepam

7.10.3. Procedure:

- Combine equal amounts of Janovsky Solution A and B in an appropriate container.
- Add a small amount of sample.
- Record any resulting color reaction(s).

7.10.4. Interpretation (color test acceptance criteria)

- A reaction which forms an initial **brown/purple color** with purple precipitate or specks indicates the possible presence of Ketamine. The purple color will intensify with heat and time.
- A reaction which forms an initial **strong purple color** and fades to **brown** indicates the possible presence of Flunitrazepam.
- The color which appears must be documented on the examination worksheet.

7.11. EHRLICH TEST

7.11.1. Reagents/Chemicals:

- p-dimethylaminobenzaldehyde
- Distilled water
- Concentrated hydrochloric acid

7.11.2. Preparation:

- Ehrlich Reagent: Dissolve 1g of p-dimethylaminobenzaldehyde to 100 mL distilled water. Add 10 mL concentrated hydrochloric acid
- Quality-test reagents with psilocybin and/or psilocin

7.11.3. Procedure:

- Combine a small amount of sample and a few drops of Ehrlich Reagent.
- Record any resulting color reaction(s).

7.11.4. Interpretation (color test acceptance criteria)

- A reaction which forms an initial **violet** color indicates the possible presence of psilocybin, psilocin, or LSD.
- The color which appears must be documented on the examination worksheet.

7.12. MAYER'S TEST

7.12.1. Reagents/Chemicals:

- Mercuric chloride
- Distilled water
- Potassium Iodide
- 10% Hydrochloric Acid (or acetic acid)

7.12.2. Preparation:

- Mayer's Reagent: Dissolve 1g of mercuric chloride in 100 mL distilled water. Add potassium iodide to dissolve scarlet precipitate.
- Quality-test reagents with PCP or Cocaine standard.

7.12.3. Procedure:

- Combine a small amount of sample and approximately 0.5 mL 10% HCl or acetic acid

- Add 1-2 drops of reagent

7.12.4. Interpretation:

- The initial formation of **white precipitate** indicates the possible presence of a tertiary amine.

8. Sampling

8.1. N/A

9. Calculations

9.1. N/A

10. Uncertainty of Measurement

10.1. N/A

11. Limitations

- 11.1. All spot tests are presumptive in nature and serve only as a guide for an analyst's analytical scheme.
- 11.2. Adulterants and complex mixtures may produce reactions that interfere with the interpretations.

12. Documentation

12.1. FCU Examination Worksheets

12.2. FCU Report of Results

13. References

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